

WO03095447

**Title:**

**PROCESS FOR THE PREPARATION OF A HYDRATE OF AN ANTHRANILIC ACID DERIVATIVE**

**Abstract:**

A hydrate of an acid addition bis-salt of an anthranilic acid derivative is produced by a process, which comprises: (a) combining, in any order, the anthranilic acid derivative, a pharmaceutically acceptable organic solvent, an excess of water and a pharmaceutically acceptable strong acid to form a mixture; (b) warming the mixture until a clear solution forms; (c) filtering the solution while it is warm, to yield a filtrate; and (d) recovering the hydrate as defined above from the filtrate. The hydrate has a defined number of moles of water of crystallisation and possesses better storage stability and dissolution characteristics than conventionally produced hydrates of such acid addition bis-salts.

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(54) Title: PROCESS FOR THE PREPARATION OF A HYDRATE OF AN ANTHRANILIC ACID DERIVATIVE

(57) Abstract: A hydrate of an acid addition bis-salt of an anthranilic acid derivative is produced by a process, which comprises: (a) combining, in any order, the anthranilic acid derivative, a pharmaceutically acceptable organic solvent, an excess of water and a pharmaceutically acceptable strong acid to form a mixture; (b) warming the mixture until a clear solution forms; (c) filtering the solution while it is warm, to yield a filtrate; and (d) recovering the hydrate as defined above from the filtrate. The hydrate has a defined number of moles of water of crystallisation and possesses better storage stability and dissolution characteristics than conventionally produced hydrates of such acid addition bis-salts.

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## PROCESS FOR THE PREPARATION OF A HYDRATE OF AN ANTHRANILIC ACID DERIVATIVE

The present invention relates to hydrated forms of acid addition salts of anthranilic acid derivatives having activity as inhibitors of P-glycoprotein (P-gp), to their preparation and to pharmaceutical and veterinary compositions containing them.

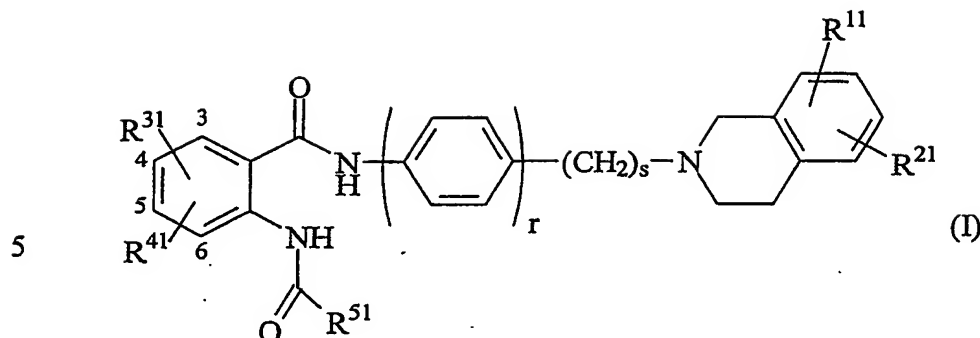
5 WO-A-98/07648 describes a series of basic compounds having structures based on anthranilic acid, and acid addition salts thereof. These compounds have activity as inhibitors of P-glycoprotein (P-gp) and may be used as modulators of multidrug resistance, for instance in overcoming the multidrug resistance of tumours and pathogens. The compounds also have potential utility in improving the absorption, distribution, metabolism  
10 and elimination characteristics of certain drugs.

Many of the compounds of WO-A-98/07648 have two basic centres and consequently form acid addition bis-salts with salt-forming acids. The bis-salts are hydrated but do not exist as specific hydrates. Rather, they are obtained as indeterminate hydrates with a variable water content which have the disadvantage of being hygroscopic.

15 It has now surprisingly been found that the addition of excess water to the system comprising the dibasic starting compound and salt-forming acid promotes the dissolution of the dibasic compound and leads to the formation of the desired acid addition bis-salt as a defined hydrate. This hydrate is stable under controlled drying conditions.

Accordingly, the present invention provides a process for producing a hydrate of  
20 an acid addition bis-salt of a compound of formula (I):

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10 wherein  $R^{11}$  and  $R^{21}$ , which may be the same or different, are each hydrogen or  $C_1$ - $C_6$  alkoxy;

$R^{31}$  and  $R^{41}$ , which may be the same or different, are each independently selected from H,  $C_1$ - $C_6$  alkyl,  $CF_3$ , a halogen,  $NH_2$ ,  $NO_2$ ,  $NHOH$ ,  $C_1$ - $C_6$  alkoxy, hydroxy and phenyl; or  $R^{31}$  and  $R^{41}$ , when situated on adjacent carbon atoms, form together with the carbon atoms to which they are attached a benzene ring or a methylenedioxy substituent,

15

$R^{51}$  is a group selected from pyridine, quinoline, isoquinoline, 5,6,7,8-tetrahydroquinoline and 5,6,7,8-tetrahydroisoquinoline, the group being unsubstituted or substituted by  $C_1$ - $C_6$  alkyl or  $C_1$ - $C_6$  alkoxy;

$r$  is 0 or 1, and

20  $s$  is 1, 2 or 3;

which process comprises:

- (a) combining, in any order, a compound of formula (I) as defined above, a pharmaceutically acceptable organic solvent, an excess of water and a pharmaceutically acceptable strong acid, to form a mixture;
- 25 (b) warming the mixture until a clear solution forms;
- (c) filtering the solution while it is warm, to yield a filtrate; and
- (d) recovering the hydrate as defined above from the filtrate.

The hydrate produced by the process of the present invention thus defined is also

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novel. Accordingly, the present invention further provides a hydrate of an acid addition salt of a compound of formula (I) as defined above, with a pharmaceutically acceptable strong acid, wherein the hydrate incorporates x moles of water of crystallisation per mole of the compound in which x is an integer of 1 to 6.

5 The integer x may be 1, 2, 3, 4, 5 or 6.

A C<sub>1</sub>-C<sub>6</sub> alkyl group may be linear or branched. A C<sub>1</sub>-C<sub>6</sub> alkyl group is typically a C<sub>1</sub>-C<sub>4</sub> alkyl group, for example a methyl, ethyl, propyl, i-propyl, -butyl, sec-butyl or tert-butyl group. A halogen is F, Cl, Br or I. Preferably it is F, Cl or Br.

10 A C<sub>1</sub>-C<sub>6</sub> alkoxy group may be linear or branched. It is typically a C<sub>1</sub>-C<sub>4</sub> alkoxy group, for example a methoxy, ethoxy, propoxy, i-propoxy, n-propoxy, n-butoxy, sec-butoxy or tert-butoxy group.

The integer s is from 1 to 3, and is preferably 1 or 2. In a preferred series of compounds of formula (I) r is 1, s is 2, R<sup>11</sup> and R<sup>21</sup> are both methoxy and R<sup>51</sup> is a quinoline or tetrahydroquinoline ring system. R<sup>51</sup> is linked via any of its available ring  
15 positions, for instance the 1-, 2-, 3- or 4-position. Typically it is linked via the 2- or 3-position. Preferably R<sup>51</sup> is a 2-quinoline or 3-quinoline group. Groups R<sup>11</sup> and R<sup>21</sup> are preferably at positions 6 and 7 of the tetrahydroisoquinoline ring system.

Examples of compounds of formula (I) are as follows:

20	Chemical Name	Compound No.
	2-Chloro-quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	1
25	4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	2
	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-thiophen-3-yl)-amide	3
30	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-4-dimethylamino-phenyl)-amide	4

	4-Hydroxy-quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	5
5	Quinoline-3-carboxylic acid (3-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-4-methyl-thiophen-2-yl)-amide	6
	Quinoline-3-carboxylic acid [2-(4-{2-[(3,4-dimethoxy-benzyl)-methyl-amino]-ethyl}-phenylcarbamoyl)-phenyl]-amide	7
10	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-4-methylsulfanyl-phenyl)-amide	8
	Quinoline-3-carboxylic acid (4-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-thiophen-3-yl)-amide	9
15	<i>N</i> -(4-{4-[2-(6,7-Dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-thiophen-3-yl)-6-methyl-nicotinamide	10
	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethylsulfanyl]-phenylcarbamoyl}-phenyl)-amide	11
	Quinoline-3-carboxylic acid (3-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-pyrazin-2-yl)-amide	12
20	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethoxy]-phenylcarbamoyl}-phenyl)-amide	13
	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-1-methyl-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	14
25	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dichloro-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	15
	Quinoline-3-carboxylic acid (2-{4-[2-(7,8-dichloro-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	16
30	Quinoline-3-carboxylic acid (2-{3-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	17
	Quinoline-3-carboxylic acid (2-{4-[2-(7-nitro-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	18
35	Quinoline-3-carboxylic acid (2-{4-[3-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-2-hydroxy-propoxy]-phenylcarbamoyl}-phenyl)-amide	19
	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-2-methyl-phenylcarbamoyl}-phenyl)-amide	20
40	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-2-methoxy-phenylcarbamoyl}-phenyl)-amide	21

	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-1-methyl-ethyl]-phenylcarbamoyl}-phenyl)-amide	22
5	Quinoline-3-carboxylic acid (2-{3-[3-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-propyl]-phenylcarbamoyl}-phenyl)-amide	23
	Quinoline-3-carboxylic acid (2-{4-[2-(7,8-dihydro-5 <i>H</i> -[1,3]dioxolo[4,5- <i>g</i> ]isoquinolin-6-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	24
10	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-diethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	25
	Quinoline-3-carboxylic acid (6-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-thieno[2,3- <i>b</i> ]pyrazin-7-yl)-amide	26
15	Isoquinoline-1-carboxylic acid {2-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethylcarbamoyl]-phenyl}-amide	27
	Quinoline-2-carboxylic acid {2-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethylcarbamoyl]-phenyl}-amide	28
	Isoquinoline-3-carboxylic acid {2-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethylcarbamoyl]-phenyl}-amide	29
20	Quinoline-3-carboxylic acid {2-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethylcarbamoyl]-phenyl}-amide	30
	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	31
25	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-5-fluoro-phenyl)-amide	32
	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-4-fluoro-phenyl)-amide	33
30	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-4,5-dimethoxy-phenyl)-amide	34
	Quinoline-3-carboxylic acid (6-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-benzo[1,3]dioxol-5-yl)-amide	35
35	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-5-nitro-phenyl)-amide	36
40	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-4-methyl-phenyl)-amide	37

	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-5-methyl-phenyl)-amide	38
5	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-4-chloro-phenyl)-amide	39
	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-5-chloro-phenyl)-amide	40
10	Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-5-amino-phenyl)-amide	41
	Quinoline-2-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	42
15	5,6,7,8-Tetrahydroquinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	43
	Quinoline-3-carboxylic acid (2-{4-[2-(3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide	44
20	Quinoline-3-carboxylic acid {2-[4-(6,7-dimethoxy-3,4-dihydro-1 <i>H</i> -isoquinolin-2-yl)methyl]-phenylcarbamoyl}-phenyl}-amide	45

The preparation of the compounds of formula (I) is described in WO-A-98/17648.

25 The pharmaceutically acceptable strong acid used in the process of the present invention is an acid which is capable of forming a salt with the two basic centres in the compounds of formula (I). These are the tetrahydroisoquinoline nitrogen atom and the nitrogen atom in the heterocyclic group R<sup>51</sup>. The pK<sub>a</sub> values of these two centres can differ significantly and the acid must be strong enough to protonate both. Examples of

30 strong acids suitable for use in the process of the present invention include arylsulphonic acids (such as toluene para-sulphonic acid), alkylsulphonic acids (such as methane sulphonic acid), hydrochloric acid and organic dicarboxylic acids such as malonic acid and succinic acid.

The pharmaceutically acceptable organic solvent used in the process of the

35 invention is typically an alcohol such as ethanol, n-propanol, isopropanol, benzyl alcohol or



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propylene glycol.

The excess water used in the process of the invention may be introduced into the reaction mixture in step (a) either separately from, or together with, the pharmaceutically acceptable organic solvent. For instance, an aqueous alcohol solution provides both the organic solvent and the water simultaneously. A suitable aqueous alcohol solution contains a 3:1 (v/v) ratio of alcohol to water. A 75% ethanol solution is particularly preferred. The water used in the process of the invention is typically demineralised water. For drug regulatory purposes the water is more preferably purified water.

In the context of the process of the invention the term "excess of water" means sufficient water both to solubilise the acid addition bis-salt of the compound of formula (I) and to achieve the optimum level of hydration of the bis-salt. An excess of water therefore denotes more than a molar excess relative to the number of moles of water of crystallisation in the final hydrate.

The level of hydration is the number of moles of water of crystallisation in the bis-salt and is an integer from 1 to 6. It may be 1, 2, 3, 4, 5 or 6. The hydrate of the invention thus typically possesses a substantially integral number of moles of water of crystallisation. The level of hydration in the solid state depends on factors including the structure of the compound of formula (I) and its capacity, *inter alia*, for forming attachments to water molecules, for instance via hydrogen bonds.

In step (b) of the process of the invention the mixture is heated until a clear solution forms. This typically entails heating the mixture to a temperature of from 35°C to the reflux temperature of the organic solvent; for instance to a temperature of from 35° to 70°C, more preferably from 45°C to 60°C.

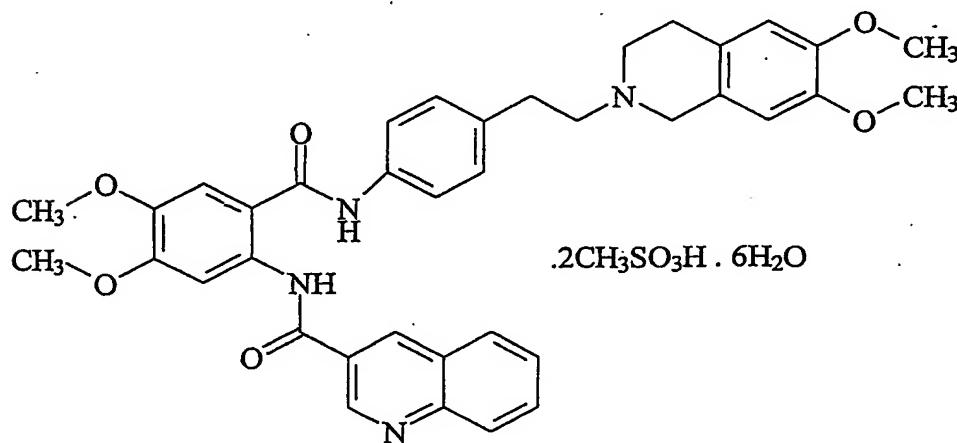
In step (c) of the process the solution is filtered while it is warm. This means that the solution is filtered whilst being held at a sufficient temperature to avoid premature precipitation of the desired product. This is typically achieved using warmed glassware, for instance glassware which is maintained at or above the temperature of the solution formed in step (b).

5 solvent is acetone. In one embodiment of the process of the invention step (d) is therefore carried out by adding the warm filtrate produced in step (c) to refluxing acetone, preferably refluxing pre- filtered acetone.

The process of the present invention is preferably carried out in an atmosphere having a relative humidity of from 40% to 80%, more preferably from 50% to 80%.

10           The hydrate produced by the process of the present invention has been shown in  
hygroscopicity studies to lose water on heating *in vacuo*, but it can be restored to its  
original level of hydration in a moist atmosphere. The hydrate has greater storage stability  
(e.g. shelf life) and better dissolution characteristics (i.e. a higher rate of dissolution) in  
pharmaceutical formulation media than the conventional indeterminate hydrates obtained by  
15   previous processes.

The hydrate of the present invention is preferably the hexahydrate of the bismesylate of quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy- 3,4-dihydro-1H-



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isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-4,5-dimethoxyphenyl)-amide. This compound, which is compound 34 in the above table, has the following structure:

The above hexahydrate is preferably prepared by a process which comprises:

- (a') combining, with warming, quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-4,5-dimethoxy-phenyl)-amide, 5 ethanol and excess water, and adding methanesulphonic acid to the mixture;
- (b') warming the mixture until a clear solution forms;
- (c') filtering the solution while it is warm, to form a filtrate; and
- (d') recovering the desired hexahydrate from the filtrate.

10 In step (a') an aqueous ethanol solution may be used. Alternatively, absolute ethanol and water may be introduced separately into the reaction system. The volume ratio of ethanol to water in either case is preferably about 3:1. The water is typically demineralised water.

In step (b') the mixture is typically warmed to a temperature of from 35°C to 15 reflux, preferably about 55°C.

In step (c') the solution is preferably filtered through warm glassware and washed through with a mixture of ethanol and water, typically into a dropping funnel maintained at about the temperature of the filtrate.

Step (d') is typically carried out by adding the filtrate to a stirred refluxing anti- 20 solvent, preferably filtered acetone. The resultant suspension is then refluxed for several hours, cooled and the hexahydrate is then collected as a solid.

Another specific example of a hydrate of the present invention is the monohydrate of the bismesylate of compound 31, namely the monohydrate of the bismesylate of quinoline-3-carboxylic acid (2-{4-{2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)- 25 ethyl}-phenylcarbamoyl}-phenyl)amide.

The hydrates of the present invention provide a convenient means for formulating the bis-salts of the compounds of formula (I) into pharmaceutical compositions. Preferred composition are liquid compositions for oral or parenteral delivery. The hydrates may thus

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be used in all the pharmaceutical applications envisaged for the compounds of formula (I) and their salts as described in WO-A-98/07648. These applications are discussed below.

Cancer cells which exhibit multi-drug resistance, referred to as MDR cells, display a reduction in intracellular drug accumulation compared with the corresponding drug-sensitive cells. Studies using in vitro derived MDR cell lines have shown that MDR is often associated with increased expression of a plasma membrane glycoprotein (P-gp) which has drug binding properties. P-gp is thought to function as an efflux pump for many hydrophobic compounds, and transfection studies using cloned P-gp have shown that its overexpression can confer the MDR phenotype on cells: see, for example, Ann. Rev. Biochem 58 137-171 (1989).

A major function of P-gp in normal tissues is to export intracellular toxins from the cell. There is evidence to suggest that overexpression of P-gp may play a clinical role in multi-drug resistance. Increased levels of P-gp mRNA or protein have been detected in many forms of human cancers - leukaemias, lymphomas, sarcomas and carcinomas. Indeed, in some cases P-gp levels have been found to be increased in tumour biopsies obtained after relapse from chemotherapy.

Inhibition of P-gp function in P-gp mediated MDR has been shown to lead to a net accumulation of anti-cancer agent in the cells. For example, Verapamil a known calcium channel blocker was shown to sensitise MDR cells to Vinca alkaloids in vitro and in vivo: Cancer Res., 41, 1967-1972 (1981). The proposed mechanism of action involves competition with the anti-cancer agent for binding to the P-gp. A range of structurally unrelated resistance-modifying agents acting by this mechanism have been described such as tamoxifen (Nolvadex:ICI) and related compounds, and cyclosporin A and derivatives.

Compounds of formula I as defined above and their pharmaceutically acceptable salts have been found in biological tests to have activity as inhibitors of P-gp. They can be used to modulate MDR, in particular P-gp mediated MDR. The results are set out in Example 1 which follows. As P-gp inhibitors the compounds may be used as multi-drug resistance modifying agents, also termed resistance-modifying agents, or RMAs. The

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compounds can modulate, e.g. reduce, or eliminate multi-drug resistance, especially that which is P-gp mediated.

The compounds can be used in a method of potentiating the cytotoxicity of an agent which is cytotoxic to a tumour cell. Such a method comprises, for instance, 5 administering one of the compounds to the tumour cell whilst the tumour cell is exposed to the cytotoxic agent in question. The therapeutic effect of a chemotherapeutic, or antineoplastic, agent may thus be enhanced. The multi-drug resistance of a tumour cell to a cytotoxic agent during chemotherapy may be reduced or eliminated.

The compounds can also be used in a method of treating a disease in which the 10 responsible pathogen exhibits multi-drug resistance, especially P-gp mediated multi-drug resistance for instance multi-drug resistant forms of malaria (Plasmodium falciparum), tuberculosis, leishmaniasis and amoebic dysentery. Such a method comprises, for instance, administering one of the compounds with (separately, simultaneously or sequentially) the drug to which the pathogen concerned exhibits multi-drug resistance. The therapeutic 15 effect of a drug directed against a multidrug resistant pathogen may thus be potentiated.

A human or animal patient harbouring a tumour may be treated for resistance to a chemotherapeutic agent by a method comprising the administration thereto of one of the compounds of formula (I) as defined above. The compound is administered in an amount effective to potentiate the cytotoxicity of the said chemotherapeutic agent. Examples of 20 chemotherapeutic or antineoplastic agents which are preferred in the context of the present invention include Vinca alkaloids such as vincristine and vinblastine; anthracycline antibiotics such as daunorubicin and doxorubicin; mitoxantrone; actinomycin D; taxanes e.g. taxol; epipodophyllotoxins e.g. etoposide and plicamycin.

The compounds of formula (I) as defined above may also be used in a method of 25 enhancing the absorption, distribution, metabolism and/or elimination characteristics of a therapeutic agent, which method comprises administering to a patient, separately, simultaneously or sequentially, one of the compounds and the said therapeutic agent. In particular this method may be used to enhance the penetration of the therapeutic agent into

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the central nervous system, or to enhance the oral absorption of the therapeutic agent.

For instance, the compounds can be used in a method of facilitating the delivery of drugs across the blood brain barrier, and in the treatment of AIDS or AIDS related complex. A human or animal patient in need of such treatment may be treated by a  
5 method comprising the administration thereto of one of the present compounds.

The hydrates of the present invention can be formulated for administration in a variety of dosage forms, for example orally such as in the form of liquid solutions or suspensions or parenterally, for example intramuscularly, intravenously or subcutaneously. The present compounds may therefore be given by injection or infusion.

10 The dosage depends on a variety of factors including the age, weight and condition of the patient and the route of administration. Typically, however, the dosage adopted for each route of administration is such as to achieve the delivery of from 0.001 to 50 mg/kg body weight, most commonly in the range of 0.01 to 5 mg/kg, of the compound of formula (I). Such a dosage may be given, for example, from 1 to 5 times daily by bolus infusion,  
15 infusion over several hours and/or repeated administration.

The hydrates of the present invention are formulated for use as a pharmaceutical or veterinary composition also comprising a pharmaceutically or veterinarily acceptable carrier or diluent. The compositions are typically prepared following conventional methods and are administered in a pharmaceutically or veterinarily suitable form. An agent for use  
20 as a modulator of multi-drug resistance comprising a hydrate of the present invention is therefore provided.

The present hydrates may be administered in any conventional form, for instance as follows:

A) Orally, for example, as aqueous or oily suspensions, liquid solutions, emulsions,  
25 syrups or elixirs. Compositions intended for oral use may be prepared according to any method known in the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant



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The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oils; or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally occurring phosphatides, for example soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan mono-oleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavouring agents. Syrups and elixirs may be formulated with sweetening agents, for example glycerol, sorbitol or sucrose. In particular a syrup for diabetic patients can contain as carriers only products, for example sorbitol, which do not metabolise to glucose or which only metabolise a very small amount to glucose.

Such formulations may also contain a demulcent, a preservative and flavouring and coloring agents.

15 B) Parenterally, either subcutaneously, or intravenously, or intramuscularly, or intrasternally, or by infusion techniques, in the form of sterile injectable aqueous or oleaginous suspensions. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic pharmaceutically-acceptable diluent or solvent, for example as a solution in 1,3-butane diol.

25 Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition fatty acids such as oleic acid find use in the preparation of injectables;

C) By inhalation, in the form of aerosols or solutions for nebulizers;

D) Rectally, in the form of suppositories prepared by mixing the drug with a



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suitable non-irritating excipient which is solid at ordinary temperature but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and poly-ethylene glycols;

E) Topically, in the form of creams, ointments, jellies, collyriums, solutions or  
5 suspensions.

Daily dosages can vary within wide limits and will be adjusted to the individual requirements in each particular case. In general, for administration to adults, an appropriate daily dosage is in the range of about 5 mg to about 500 mg of the compound of formula (I), although the upper limit may be exceeded if expedient. The daily dosage can  
10 be administered as a single dosage or in divided dosages.

The invention will be further described in the Examples which follow:

**Example 1: Preparation of the bismesylate hexahydrate of compound 34**

15 Quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethyl]-phenylcarbonyl}-4,5-dimethoxy-phenyl)-amide (10.33g, 16mmol) was stirred in a mixture of ethanol (62 ml, 6 volumes) and demineralised water (20.6 ml, 2 volumes) and methanesulphonic acid (3.38g, 35.2 mmol) was added. The mixture was heated to 55°C to give a clear orange coloured solution and filtered under  
20 vacuum through a preheated funnel into a dropping funnel maintained at about 55°C. This was followed by a 1:1 wash of ethanol and demineralised water (2 x 20 ml, 4 volumes) at about 55°C.

The combined filtrate and washes were added over about 20 minutes to stirred, refluxing acetone (310 ml, 30 volumes) to give a sticky yellow solid. After refluxing the  
25 mixture for a further 1 hour, the yellow suspension was cooled for 2 hours in an ice-bath to about -5°C. The product was collected by filtration, washed with filtered acetone (3 x 50 ml) and pulled dry for about 30 minutes. The pale yellow solid was transferred to an open dish and allowed to dry overnight in a gentle current of air at ambient temperature and

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humidity to give the title compound.

Weight: 13.86g; yield: 91.5% theory for hexahydrate.

The water content by Karl Fischer was 12.54% (theory for hexahydrate : 11.41%).

5 The ratio of methanesulphonic acid to base by  $^1\text{H}$  NMR was 1.93 : 1 ( theory requires 2:1).

The purity by HPLC was 99.7% a/a.

Elemental analyses were consistent with  $\text{C}_{38}\text{H}_{38}\text{N}_4\text{O}_6 \cdot 2\text{CH}_3\text{SO}_3\text{H}$ , 12.54% water.

Found: C= 50.35%; H= 6.15%; N= 5.85%; S=6.71%. [ $\text{C}_{40}\text{H}_{46}\text{N}_4\text{O}_{12}\text{S}_2$ ,  
10 12.54%  $\text{H}_2\text{O}$  requires C= 50.09%; H= 6.24%; N= 5.84%; S= 6.68%.]

**Example 2: Preparation of the bismesylate monohydrate of compound 31**

A suspension of quinoline-3-carboxylic acid (2-{4-{2-(6,7-dimethoxy-3,4-  
15 dihydro-1H-isoquinolin-2-yl)-ethyl]-phenylcarbamoyl}-phenyl)-amide (3.0g, 5.11 mmol)  
in a mixture of ethanol (18 ml, 6 volumes) and water (6 ml, 2 volumes) was warmed to  
about 50°C and methane sulphonic acid (1.08g, 11.2 mmol) was added to give a pale  
orange coloured solution. This was filtered and added rapidly to stirred, refluxing acetone  
(60 ml, 20 volumes). The flask and filter were washed with a 1 : 1 mixture of ethanol  
20 and demineralised water (6 ml, 2 volumes) at about 50°C and this was added to the  
refluxing acetone. After refluxing the mixture for 2 hours, the suspension was cooled to  
ambient temperature and the pale yellow product was collected by filtration, washed with  
acetone (9 ml) and dried in vacuum at ambient temperature.

The yield was 3.8g (93.3% for monohydrate).

25 The water content, by Karl Fischer, was 2.42% (theory for monohydrate: 2.26%).

The purity by HPLC was 100 % a/a.

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**Example 3: Hygroscopicity and dehydration/re-hydration study**

The hexahydrate produced in Example 1 was analysed as follows. Four  
5 desiccator cabinets were prepared to contain open dishes of the following saturated salt  
solutions, to achieve the following approximate relative humidities (RH).

	<u>% RH at Ambient Temp.</u>	<u>Saturated Salt Solution</u>
	33%	MgCl <sub>2</sub> .6H <sub>2</sub> O
10	60%	NaBr
	75%	NaCl
	95%	KNO <sub>3</sub>

Dehydration of samples was carried out by storing them over silica gel or over  
15 P<sub>2</sub>O<sub>5</sub> at reduced pressure (65mm Hg). All studies were performed at ambient laboratory  
temperature (15-22°C).

**Hygroscopicity study**

Pre-equilibrated weighing bottles and lids (stored at 33% RH for a minimum of 24  
20 hours) were weighed on a tared 5-figure analytical balance, and the weight recorded. To  
these the hydrate was added (approx 2g), the weight recorded and the difference (the  
accurate weight of the drug substance) calculated. The weighing bottles, lids and contents  
were then transferred to the 33% RH cabinet, with the lids removed and placed to the  
side.

25 At appropriate times, the lids were replaced and the weight measured. After  
weighing, the samples were gently agitated and placed back in the 33%RH cabinet, again  
with the lid removed and placed to one side. This process continued until all % weight  
changes for all samples were seen to have reached stable end-points. When stable end-

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points. When stable end-points were observed, the samples were stepped up to the next %RH cabinet by transferring the drug to pre-equilibrated weighing bottles, recording their weight as before. Samples were stored progressively at 33%RH, 60%RH, 75%RH and 95%RH.

5

#### Dehydration/Re-hydration study

During this study, samples were dispensed to pre-equilibrated weighing bottles as in the hygroscopicity study, and were then stored over silica gel without reduced pressure, then over silica gel under reduced pressure (65mm Hg) to dehydrate the hydrate. Once a  
10 stable weight was achieved over  $P_2O_5$ , samples were re-hydrated by storing them at 33%RH to 95%RH, in parallel with the hygroscopicity study as described above.

#### Determination of water content

Water contents were measured by coulometric KF (Karl Fischer) titration.

15

#### IR analysis

Samples of the hydrate were submitted for IR analysis (ATR) at the end of the dehydration study and at the ends of the re-hydration and hygroscopicity studies.

### 20 Results

#### Weight Change

Results for the hygroscopicity study and the dehydration/re-hydration study are shown in Table 1.

25

#### Table 1 Dehydration/Rehydration and Hygroscopicity Results up to 60% RH

(Results in brackets are KF moisture results, % w/w)

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	Condition	Cumulative time (h)	Cumulative % change	
			Dehydration/ Rehydration	Hygroscopicity*
5	silica, no vacuum.	3.00	(12.53) - 2.29	
		8.50	-3.58	
		24.50	-5.92	
		30.50	-6.39	
		56.00	-8.06	
		80.00	-8.50	
10		237.50	-8.96	
		288.50	-9.07	
	silica, with vacuum.	308.00	-9.07	
15		401.00	-8.76	
	P <sub>2</sub> O <sub>5</sub> with vacuum	425.00	-10.85	
		452.00	-11.00	
		476.75	-11.19	
		570.25	-11.43	
		641.00	-11.35	
		760.50	-11.51	
		904.25	-11.46	
		1071.00	-11.54	
20		1144.00	-11.48	
	33% RH	1147.25	(3.82) -6.61	(12.45) -0.17*
		1150.75	-4.80	-0.22
		1168.00	-2.47	-0.47
		1240.75	-0.68	-0.37
		1268.50	-0.62	-0.42

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		1312.50	-0.62	-0.49
		1431.25	-0.83	-0.63
		1502.25	-0.66	-0.51
		1672.50	-0.91	-0.70
5		1741.50	-0.97	-0.76
	60% RH	1744.50	0.19	0.12
		1746.50	0.31	0.13
		1762.50	0.30	0.08
		1835.75	0.54	0.27
10		1932.25	0.64	0.37
		2101.50	0.86	0.56
		2147.75	0.62	0.34
		2273.75	0.61	0.34
		2410.25	0.51	0.27
15	75% RH	2417.25	1.18	0.87
		2434.25	1.21	0.88
		2441.25	1.26	0.88
		2482.00	1.23	0.92
		2577.75	1.35	1.00
20		2609.75	1.38	0.96
		2681.25	1.41	1.08
		2770.25	1.36	1.01
		2817.75	1.44	1.09
	95% RH	2825.25	2.28	1.85
25		2841.75	2.73	2.23
		2915.75	3.06	2.57
		2937.75	3.21	2.71

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	2991.25	3.25	2.82
	3082.25	3.86	3.28
	3130.00	4.12	3.51
	3182.25	3.99	3.39
5	3252.25	4.19	3.62
	3350.25	4.22	3.65
	3422.25	4.35	3.64
	3636.25	5.31	4.12
10	4332.75	(15.81) 5.58	(15.35) 4.45

\* The actual cumulative storage time for the hygroscopicity sample is the tabulated value minus 1144 hours.

#### IR Results

15 IR spectra for the samples taken at the end of the re-hydration and hygroscopicity studies showed no significant differences in the wavenumber and relative intensities of the main bands.

The IR spectrum of the sample taken at the end of the dehydration study showed the expected loss of the major, broad water band at about  $3500\text{ cm}^{-1}$ . Other than this the spectrum was similar to those recorded after the re-hydration and hygroscopicity studies with some small differences in relative intensities and resolution. The main differences in relative intensity occurred at about  $1650$ ,  $1200$  and  $850\text{ cm}^{-1}$ .

#### Conclusion

25

Dehydration of the hydrate of Example 1 over increasing desiccating conditions results in a relatively rapid loss of water. The final weight change (-11.5%) is similar to the initial water content of the sample (12.5%). Re-hydration at

10 From the data generated the behaviour of the material is in line with its existing as a hexahydrate. The theoretical water content for the hydrate of Example 1 is 11.4 %w/w. Thus a water content of about 12.5%w/w, the initial value before dehydration, could correspond to a damp hexahydrate. (A water content of 12.5%w/w would correspond to 6.7 moles of water per mole of bismesylate).

The results thus indicate that the hydrate of Example 1 is a stable hexahydrate with a small amount of additional, loosely bound water. In this hydrated form, the material does not display the hygroscopic properties exhibited by the bismesylate of compound 34 produced by conventional techniques, for instance as described in WO98/07648.

**Example 4:**     **Testing of compounds of formula (I) and their salts as**  
**modulators of MDR**



### Materials and Methods

The EMT6 mouse mammary carcinoma cell line and the MDR resistant subline AR 1.0 were cultured in RPMI 1640 medium containing 10% foetal calf serum and 2mM glutamine at 37°C in 5% CO<sub>2</sub>. Cells were passaged between 1 in 200 and 1 in 2000 in the case of the parental cell line and between 1 in 20 and 1 in 200 in the case of the MDR resistant subline, after trypsinisation (0.25% trypsin, 0.2gl<sup>-1</sup>, EDTA).

#### 1. Drug accumulation assay

AR 1.0 cells were seeded 48 hours prior to assay into 96 well opaque culture plates (Canberra Packard). The assay medium contained a mixture of tritiated Daunorubicin (DNR) (0.3 mCi/MI), a cytotoxic agent, and unlabelled DNR (2mM). Compounds of formula I were serially diluted in assay medium over a range of concentrations from 0.508 nM to 10 mM. The cells were incubated at 37°C for 1 hr before washing and determination of cell associated radioactivity. Results are expressed as an IC<sub>50</sub> for accumulation where 100% accumulation is that observed in the presence of the known RMA verapamil at a concentration of 100 mM.

The results are set out in the following Table A.

**TABLE A**

Compound No.	IC <sub>50</sub> (mM) Accumulation
1	0.425
2	>10
3	0.087
4	0.37
5	>10
6	0.431
7	0.098
8	0.213

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	9	0.113
	10	0.203
	11	0.453
	12	0.207
5	13	1.89
	14	0.347
	15	2.27
	16	>10
	17	0.593
10	18	6.955
	19	0.038
	20	0.061
	21	0.071
	22	0.135
15	23	6.424
	24	1.679
	25	0.389
	26	8.672
	27	2.0
20	28	1.2
	29	1.8
	30	10
	31	0.05
	32	0.022
25	33	0.019
	34	0.064
	35	0.084
	36	0.015
	37	0.36
30	38	0.094
	39	0.014
	40	0.18
	41	1.0
	42	0.8

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43	0.097
44	0.32
45	0.04

## 5 2. Potentialiation of Doxorubicin toxicity

(a) Selected compounds of formula (I) were examined for their ability to potentiate the toxicity of doxorubicin in AR 1.0 cells. In initial proliferation assays compounds were titrated against a fixed concentration of doxorubicin (0.34 mM) which alone is non-toxic to AR 1.0 cells. After a four day incubation with doxorubicin proliferation was measured using the colorimetric sulphorhodamine B assay (Skehan *et al*; J Natl. Cancer Inst. 82 pp 1107-1112 (1990)). The results are shown in Table B.

(b) Cells were cultured for four days with a titration of doxorubicin (0.263 nM - 17.24 mM) in the presence of a fixed concentration of each compound. Proliferation was quantified as described by Skehan *et al*, *loc cit*. The IC<sub>50</sub> (concentration required to reduce proliferation to 50% of the untreated controls) for doxorubicin alone and with each compound were derived and used to calculate the potentiation index (PI):

$$\text{PI} = \frac{\text{IC}_{50} \text{ for Doxorubicin alone}}{\text{IC}_{50} \text{ for Doxorubicin plus RMA}}$$

The results are shown in Tables C1 and C2.

### TABLE B

Compound No.	Compound Toxicity (IC <sub>50</sub> mM)	Toxicity with Cytotoxic Agent (IC <sub>50</sub> mM)
27	40	1.0
28	40	0.55
29	30	0.3
33	0.32	0.005

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5	34	0.93	0.0018
	35	0.9	0.0014
	36	0.31	0.0038
	37	8.6	0.015
	38	6.7	0.005
	39	7.0	0.005
	40	7.4	0.04
	41	36.8	4.4
	42	1.7	0.07
10	43	9.5	0.05
	44	7.7	0.00035
	45	9.2	0.022

15

**TABLE C1**

	Compound No.	Potentiation Index at RMA Concentration				
		100 nM	50nM	30 nM	20 nM	10 nM
20	3	601	307	159		11
	4	45	2.99	1.93		1.45
	6	68	19	7.4	3.4	1.4
		171	149	95		11
	7	168	97	35		3
25	8	175	85	23		2
	9	185	143	142		13
	10	81	15	4		1.5
	11	25	4.4	1.6	1.3	1.0
	12	79	46	15	8	1.8
30	14	60	7	4		1
	17	13.7	3.4	1.3		1.0
	19			34		16
	20	33	14	3		3
	21	2.2	1.1			

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23	1.4	1.2	1.1		
24	116	37	1.9		1
25	50	28	7		1.4

5

**TABLE C2**

Compound No.	Potentiation Index at RMA Concentration:				
	500 nM	300 nM	100 nM	30 nM	10 nM
31		150	120	67	15
32			100	100	38
33			94	60	16
34			280	225	78
35				188	43
36				300	90
37				36	2.1
38				68	6
39				57	6
40				6	5
41				1	1
44			112	18	2.2
45				7.2	1.3

25

### 3. Potentiation of toxicity of various cytotoxic agents

The potentiation indices of a selection of compounds using a variety of cell lines and a variety of cytotoxics other than doxorubicin were measured following the protocol described above for doxorubicin, and the results are shown in Table D.

30

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**TABLE D**

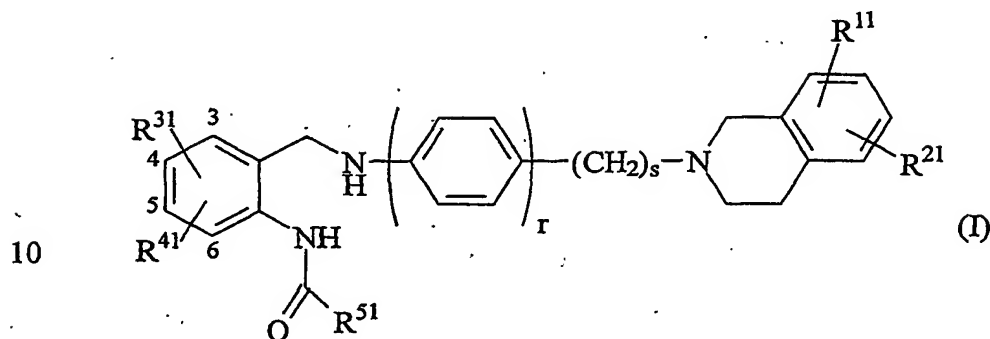
			Potentiaton Index at RMA Concentration		
Compound No.	Cell line	Cytotoxic	50 nM	30 nM	10 nM
5 3	2780AD	Taxol	1126	425	18
3	H69/LX4	Vincristine	356	79	2
3	AR 1.0	Taxol	407	308	50
6	H69/LX4	Taxol	9	3	1
7	H69/LX4	Taxol	877	236	2.2
10 34	AR 1.0	Etoposide	51	45	26

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CLAIMS

1. A process for producing a hydrate of an acid addition salt of a compound of formula (I):

5



15 wherein  $R^{11}$  and  $R^{21}$ , which may be the same or different, are each hydrogen or  $C_1-C_6$  alkoxy;

$R^{31}$  and  $R^{41}$ , which may be the same or different, are each independently selected from H,  $C_1-C_6$  alkyl,  $CF_3$ , a halogen,  $NH_2$ ,  $NO_2$ ,  $NHOH$ ,  $C_1-C_6$  alkoxy, hydroxy and phenyl; or  $R^{31}$  and  $R^{41}$ , when situated on adjacent carbon atoms, form together with the

20 carbon atoms to which they are attached a benzene ring or a methylenedioxy substituent;

$R^{51}$  is a group selected from pyridine, quinoline, isoquinoline, 5,6,7,8-tetrahydroquinoline and 5,6,7,8-tetrahydroisoquinoline, the group being unsubstituted or substituted by  $C_1-C_6$  alkyl or  $C_1-C_6$  alkoxy;

25 r is 0 or 1, and

s is 1, 2 or 3;

which process comprises:

(a) combining, in any order, a compound of formula (I) as defined above, a

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pharmaceutically acceptable organic solvent, an excess of water and a pharmaceutically acceptable strong acid to form a mixture;

- (b) warming the mixture until a clear solution forms;
- (c) filtering the solution while it is warm, to yield a filtrate; and
- 5 (d) recovering the hydrate as defined above from the filtrate.

2. A process according to claim 1 wherein the hydrate is recovered by diluting the filtrate with an anti-solvent.

10 3. A process according to claim 2 wherein the anti-solvent is pre-filtered acetone.

4. A process according to any one of the preceding claims wherein the pharmaceutically acceptable solvent is an alcohol.

15 5. A process according to claim 4 wherein the alcohol is selected from ethanol, n-propanol, isopropanol, benzyl alcohol and propylene glycol.

6. A process according to any one of the preceding claims wherein the pharmaceutically acceptable strong acid is methanesulphonic acid.

20

7. A process according to any one of the preceding claims wherein the compound of formula (I) is quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethyl]-phenylcarbonyl}-4,5-dimethoxyphenyl) amide.

25 8. A hydrate of an acid addition salt of a compound of formula I as defined in claim 1, formed with a pharmaceutically acceptable strong acid, wherein the hydrate incorporates x moles of a water of crystallisation per mole of the compound, in which x is an integer from 1 to 6.



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9. A hydrate according to claim 8 wherein the pharmaceutically acceptable strong acid is methanesulphonic acid.
10. A hydrate according to claim 8 which is the hexahydrate of the bismesylate of  
5 quinoline-3-carboxylic acid (2-{4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethyl]-phenylcarbonyl}-4,5-dimethoxyphenyl) amide.
11. A hydrate which is obtainable by a process as defined in any one of claims 1 to 7.
- 10 12. A pharmaceutical or veterinary composition comprising a hydrate as defined in any one of claims 8 to 11 and a pharmaceutically or veterinarily acceptable carrier or diluent.
13. A hydrate as defined in any one of claims 8 to 11 for use in a method of medical treatment of the human or animal body by therapy.
- 15 14. Use of a hydrate as defined in any one of claims 8 to 11 in the manufacture of medicament for use as an inhibitor of P-glycoprotein.
15. An agent for use as an inhibitor of P-glycoprotein, which comprises a hydrate  
20 as defined in any one of claims 8 to 11.
16. A method of modulating P-gp mediated MDR in the treatment of tumours, which method comprises administering to a patient, harbouring a tumour which expresses P-gp mediated MDR, a therapeutically effective amount of a hydrate as defined in any one of  
25 claims 8 to 11.
17. A method of potentiating the cytotoxicity of an agent which is cytotoxic to a tumour cell, which method comprises administering to a patient in need thereof a

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therapeutically effective amount of a hydrate as defined in any one of claims 8 to 11.

18. A method of treating a disease in which the responsible pathogen exhibits multi-  
drug resistance, which method comprises administering to a patient in need thereof a  
5 therapeutically effective amount of a hydrate as defined in any one of claims 8 to 11.
19. A method of enhancing a characteristic of a therapeutic agent, the said  
characteristic being selected from the group consisting of penetration, absorption,  
distribution, metabolism and elimination, which method comprises administering to a patient  
10 in need thereof a therapeutically effective amount of a hydrate as defined in any one of  
claims 8 to 11.

## INTERNATIONAL SEARCH REPORT

PCT/GB 03/02060

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D401/12 A61K31/47 C07D409/14 C07D217/04

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 17648 A (ASHWORTH PHILIP ANTHONY ; SANDERSON JASON TERRY (GB); WILLIAMS SUSA) 30 April 1998 (1998-04-30) cited in the application the whole document -----	1-15



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \* & \* document member of the same patent family

Date of the actual completion of the international search

18 July 2003

Date of mailing of the international search report

28/07/2003

Name and mailing address of the ISA

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Authorized officer

Lauro, P

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

**Continuation of Box I.1**

Although claims 16-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. Although claims 16-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

# INTERNATIONAL SEARCH REPORT

PCT/GB 03/02060

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

PC1/GB 03/02060

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